

bacterial antigens characterized recently, some induce highly specific skin test results in animals. The aim of the present study is to investigate the ability of secreted antigens from *M. tuberculosis* (ESAT6, MPT63, MTC28) and an *M. avium* antigen (mig) to elicit specific human T-cell reactions in vitro

Methods: PPD and antigens from *M. tuberculosis* and *M. avium* were analyzed by lymphocyte proliferation and IFN- γ secretion of mononuclear cells obtained from immunocompetent patients (below 30 years of age) with culture-proven tuberculosis (TB, $n=15$) or *M. avium* infection (MAI, $n=6$)

Results: In TB patients, lymphocyte proliferation (SI3) was observed in 75% for ESAT6, 27% for MPT63, 50% for MTC28 and 78% for a cocktail of these antigens. Thirty-three per cent of TB patients also reacted to mig; all recognized PPD. In the MAI group, mig elicited responses in 50%, and PPD in 67%. Neither ESAT6 nor MPT63 were recognized in vitro by these patients, while MTC28 and the cocktail induced a reaction in one patient. Healthy controls showed comparable results to the MAI group, but no proliferation to PPD

Conclusions: Our data indicate that single secreted *M. tuberculosis* antigens allow differentiation of *M. tuberculosis* and *M. avium* infection with a higher specificity than PPD. However, single antigens might not be sensitive enough for clinical diagnostic purposes, where defined antigen cocktails could be of advantage

O40 Optimal in vitro growth conditions for *Mycobacterium genavense*

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Mycobacterium genavense was first described in 1990 and was accepted as a novel species in 1993. This mycobacterium is responsible for infections in animals (birds and dogs) and humans (AIDS and non-HIV individuals). *M. genavense* appears to have fastidious growth requirements when routine mycobacteriologic culture methods are used. We optimized the growth conditions for *M. genavense*, using nude mice previously infected with various *M. genavense* strains. The inhibitory effects of neutral pH, POES and PANTA in the BACTEC System were demonstrated. Optimal growth was obtained at pH 6.0 without additives. The impact of various decontamination procedures was also investigated; NALC-NaOH was the least toxic but still resulted in loss of viability of at least 100-fold. *M. genavense* was then demonstrated to be a micro-aerophilic organism, using a semi-solid medium. Furthermore, using low oxygen tensions (i.e. 2.5% or 5% O₂) rather than the standard 21% O₂ in the headspace of BACTEC bottles, very low inocula (i.e. 25 bacilli/mL) of *M. genavense* could be recovered in primary cultures. A solid medium (Middlebrook 7H 11) of acid pH and containing charcoal and blood also proved useful for the culture of *M. genavense* from clinical specimens. The micro-aerophilic nature of *M. genavense* and other mycobacteria has important implications for optimizing primary culture conditions and for the treatment of mycobacterial diseases.

Epidemiology/surveillance and outbreaks

O41 Use of microbiological studies to diagnose bacteremias, catheter-related infections and lower respiratory tract infections in ICU patients

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Objectives: To compare patterns of use of microbiological studies to diagnose bacteremia, catheter-related infections (CRIs) and lower respiratory tract infections (LRTIs) in ICU patients

Methods: Prospective surveillance (May 1996 to October 1997) of four ICUs (medical, surgical (cardiac, trauma, neurosurgery)) for the diagnosis of nosocomial infections. Registration of microbiological cultures: blood (B), catheter tip (C), tracheal secretions (T)

Results: Among 1506 patients (1019 males, 487 females; mean age 56.2 years; 11 875 patient days) the following mean infection rates were observed (infections/1000 ICU-days): bacteremia 8.3, CRI 8.2, LRTI 33.3. Diagnostic effort and yield varied among ICUs. In the medical ICU an average of 14.1 B were performed per documented bacteremia, and in the neurosurgical ICU 89. The yield of cultures of T and C also varied between units (no. T/LRTI): medical 3.5, cardiac surgery 9.7, trauma 7.9, neurosurgery 2.9 (mean 6.1). The ratios for diagnosis of CRI were (no. C/CRI): medical 9.1, cardiac surgery 16.0, trauma 9.4, neurosurgery 12.5 (mean 11.1). Cultures of B and T were performed with similar frequency during various phases of ICU stay (cultures/100 days): B mean 21.3, range 17.3–21.8; T mean 20.4, range 18.1–23.1. Cultures of C were performed more frequently during later phases of ICU stay: days 1–3, 3.4; days 4–6, 10.8; days 7–14, 18.2; day 14, 14.6. The combined diagnostic use (B+C+T/100 days) differed markedly between ICUs: medical 49.3, cardiac surgery 84.9, trauma 35.6, neurosurgery 58.1

Conclusions: Utilization of microbiological studies to diagnose common infections varied markedly between ICUs. Comparisons between units are useful to identify best performers and to educate physicians regarding the rational use of diagnostic studies.

O42 A German system for surveillance of surgical site infections (SSIs) first results and experience

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Objectives: In January 1997 a German surveillance system for surgical site infections (SSIs) was started to found a reference database of SSI rates for quality management activities

Methods: So far, 34 hospitals with 43 departments participate in the Krankenhausinfektions-Surveillance System (KISS) using the US National Nosocomial Infections Surveillance (NNIS) System protocol for SSI and the CDC definitions for nosocomial infections. Frequently performed operative procedures were selected, and for all patients who undergo one of these procedures ($n=19$), ASA score, wound classification and duration of operation are documented. From the date of the operation until discharge from the hospital, all patients are monitored for the occurrence of a wound infection

Results: Up to now, 19 951 operations with 580 SSI have been collected. The results for cholecystectomy in comparison to the NNIS data are shown below

Table 1 SSI infection rates for cholecystectomy by risk index category (NNIS, 10/86–7/97; KISS, 1/97–8/98)

	Risk-Index 0		Risk-Index 1		Risk-Index 2		Risk-Index 3	
	N	Rate	N	Rate	N	Rate	N	Rate
Cholecystectomy	1191	1.51	474	1.48	359	3.62	171	5.85
KISS	16477	0.54	5893	0.81	5554	2.25	2010	3.98

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Conclusions: These data show that a successful surveillance system that provides reliable reference data has been established in Germany. A number of quality management activities have been started in the participating hospitals, and many more hospitals would like to join the project in 1999

O43 Diphtheria in Europe

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Objectives: Within the remit of the European Commission DGXII, BioMed 2 programme (1998–2001), to form a network of Diphtheria Reference Centres within Europe, so as to improve communication and exchange of information between countries on the epidemiology and microbiology of infections caused by *Corynebacterium diphtheriae* and *C. ulcerans*

Methods: This has been achieved by the award of a DGXII grant from the European Commission, the effective collaboration of countries within and external to Europe, along with the standardization of methodologies and protocols for microbiological surveillance of diphtheria. An international quality assurance scheme for laboratory performance in diagnostic and typing methodologies has been established in order to monitor these activities

Results: The formation of Diphtheria Reference Centres within member states provides the basis for rapid exchange of information between countries and the availability of facilities for the microbiological diagnosis of diphtheria. Established centers exist in the UK and Finland, with new centers currently being established in France, Greece and Italy. Epidemiologic and molecular typing databases have also been developed for the identification and verification of new and existing strains that have the potential to cause epidemics; this is crucial for the monitoring and elimination of this resurgent disease from the European region

Conclusions: As diphtheria declines within Europe, accurate, sensitive and specific laboratory confirmation of suspect cases becomes even more important and highlights the need for countries to maintain and increase both clinical and laboratory awareness.

O44 ESBL-Ea outbreak characterization

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During a 6-week period (September to November 1997), 18 extended-spectrum β -lactamase-producing *Enterobacter aerogenes* (ESBL-Ea) strains were isolated in clinical samples from five ICU patients. Three patients developed bacteremia, one a surgical wound infection and one colonization of the respiratory tract. The prior ICU average patient stay was 27.8 days, with all patients receiving prior cephalosporin therapy. The emergence of ESBL-Ea was coincidental with a substantial increase in the use of fourth-generation cephalosporins (4GC) in previous months. All the isolates were resistant to ceftazidime, cefotaxime, aztreonam, tobramycin, ciprofloxacin, tetracycline and co-trimoxazole, and susceptible to imipenem, gentamicin

and amikacin by the microdilution method. ESBL production was detected by double disk diffusion test, but the ESBL Etest failed to detect it. Two β -lactamases of pI 6.5 and pI 8.5 were detected by isoelectric focusing analysis of enzymatic extracts. The pI 6.5 β -lactamase could be transferred by conjugation, conferring an ESBL resistance phenotype to transconjugants. The pI 8.2 β -lactamase could not be transferred; therefore, it was considered to be chromosomal. The ESBL was included in the TEM family by PCR. Genotypic characterization showed a single clone by pulsed-field gel electrophoresis, and an identical plasmid profile in all the isolates. After introduction of isolation measures and 4GC restriction, no new cases of infection or colonization by the epidemic strain were detected. The conclusion is that the clustered infections due to multiresistant Ea observed were caused by the spreading of an epidemic clone TEM-type ESBL producer, and disappeared after implementation of isolation measures and reduction of 4GC consumption.

Fungal diseases: diagnosis, virulence factors, antifungal agents

O45 Comparative evaluation of PCR and serology for diagnosis of aspergillosis in patients with hematologic malignancies

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Objectives: In this study we compared the diagnostic value of two different methods that both promise to provide early and sensitive detection of invasive aspergillosis (IA)

Methods: We used a PCR-based method which amplifies a fungus-specific 18S rRNA fragment with subsequent species-specific hybridization (according to Einsele et al; DNA extraction had been modified) as well as the Platelia *Aspergillus* antigen detection system (Sanofi Pasteur). Within a period of 18 months, blood and BAL (if available) of 34 patients with hematologic malignancies (NHL, $n=7$; AML, $n=18$; ALL, $n=3$; CML, $n=6$) were prospectively sampled once or twice a week during hospital stay and processed as described above (samples: $n=226$). Sixteen patients showed no clinical or laboratory evidence for IA (group A), and 12 patients had clinical and radiologic signs of probable IA (group B). Group C was composed of six patients with proven IA (five histologically proven, one positive in repeated BAL)

Conclusion: The Platelia test appeared to provide more conclusive results in proven IA than the PCR-based method.

O46 Diagnosis in invasive pulmonary aspergillosis, an animal model

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Objective: Two diagnostic tests, ELISA for circulating galactomannan and PCR, were compared for diagnosing and monitoring invasive pulmonary aspergillosis in an animal model

Methods: In a model of invasive left-sided pulmonary aspergillosis in persistently neutropenic rats, dissection was performed on day 1, day 3, day 5 and day 7 ($n=20$ each day) after inoculation. Within blood samples, PCR as well as ELISA was performed. Fungal